Unraveling the effects of plant hydraulics on stomatal closure during water stress in walnut.

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Abstract

The objectives of the study was to identify the relevant hydraulics parameters associated with stomatal regulation during water stress and to test the hypothesis of a stomatal control of xylem embolism on walnut trees (*Juglans regia x nigra*). The hydraulic characteristics of the sap pathway have been experimentally altered with different methods in order to induce decreases in transpiration (E_{plant}) and stomatal conductance (g_s). In a first set of experiments, potted trees were exposed to a soil water depletion to alter soil water potential (Ψ_{soil}), soil (R_{soil}) and root hydraulic resistances (R_{root}). In a second set of experiments, soil temperature was changed to alter R_{root} alone. In a third experiment, shoot resistance (R_{shoot}) was increased by inducing embolism in the trunk with a pressure chamber. Stomata closed in response to these stresses with the effect of maintaining the xylem water potential (Ψ_{xyl}) above -1.4 MPa. The same dependence of E_{plant} and g_s on Ψ_{xyl} was observed for all the experiments. This suggested that stomata were not responding to changes in Ψ_{soil}, R_{soil}, R_{root}, or R_{shoot} *per se* but rather to their impact on Ψ_{xyl}. The vulnerability to xylem embolism of the different organs along the sap pathway has been characterized. Vessels in the leaf rachises were the most vulnerable, with a threshold xylem water potential value of -1.4 MPa, corresponding precisely to the minimum Ψ_{xyl} value noticed in the previous experiments. Theoretical calculations further suggested that if the dependence of E_{plant} on Ψ_{xyl} was changed, embolism would have developed in the leaf rachis. This suggested that Ψ_{xyl} was the physiological parameter regulated by the stomatal closure during water stress, which had the effect of preventing the extensive development of cavitation during water stress.

Key words:

Drought stress – Hydraulic conductance - Stomatal regulation – Water relations – Water potential - Xylem embolism – *Juglans*

Running title: Cavitation avoidance in walnut

Abbreviations and symbols:

D = air vapor pressure deficit; E_{plant} = plant transpiration rate ; E_{max} = maximum value of E_{plant}; g_s = stomatal conductance to water vapor ; K_{init} , K_{max} = initial and maximal hydraulic conductance of xylem segments; PLC = Percentage of loss of hydraulic conductance; R = hydraulic resistance; R_{root} = root R; R_{shoot} = shoot R; R_{plant} = whole plant R; T_{soil} = soil temperature; SF_{plant} = plant leaf area; VC = vulnerability curve; Ψ = water potential; Ψ_{xyl} = xylem Ψ; Ψ_{leaf} = leaf Ψ; Ψ_{soil} = soil Ψ;

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Introduction

Drought is one of the most limiting environmental stresses for plant production (Kramer and Boyer 1995). Plants respond to drought by closing their stomata, which reduces leaf transpiration and prevents the development of excessive water deficits in their tissues. The drawback of the stomatal closure for plants is that their carbon gain is lowered and their growth is impaired. Therefore, information about plant responses to drought and their underlying mechanisms will have relevance for plant adaptation to new climatic conditions and breeding programs (Schulze et al. 1987).

Many physiological parameters are known to be implicated in the stomatal regulation, some of these parameters been involved in the mechanics of stomatal movements, other in the signals triggering these movements (Zeiger et al. 1987). A new set of parameters has been proposed in the recent literature, all being related to the hydraulics of plants. Indeed, it has been suggested that stomata may respond to drought-induced changes in the hydraulic characteristics of the sap pathway, such as whole plant hydraulic resistance (R_{plant}) (Meinzer and Grantz 1990; Cochard et al. 1996; Lu et al. 1996), soil (R_{soil}) and root resistance (R_{root}) (Meinzer and Grantz 1990; Cochard et al. 2000) or shoot resistance (R_{shoot}) (Salleo et al. 1992; Sperry et al. 1993). However, during water stress, changes in the above parameters are not independent and, further, correlated to variations in soil water potential (Ψ_{soil}), whose impact on stomatal function as long been recognized (e.g. Hinckley and Bruckerhoff 1975). A first objective of our study was to experimentally uncouple R_{soil}, R_{root}, R_{shoot}, and Ψ_{soil} in order to identify the specific hydraulic parameters associated with stomatal regulation during water stress. The experiments were conducted on potted walnut trees in a growth cabinet to minimize the effects of temperature, light intensity, air vapor deficit and air CO^2 concentration on stomatal behavior.

It has also been suggested that stomatal closure during water stress might be associated with the maintain of xylem integrity (Tyree and Sperry 1988; Jones and Sutherland 1991). Indeed, sap is transported under negative pressures in plants, and, therefore, is subjected to cavitation events (Pickard 1981). Cavitation occurs when the negative sap pressure exceeds a threshold value defined by anatomical characteristics (Sperry and Tyree 1988). Many species have been found to operate very close to the point of embolism. Therefore, stomata control both plant water losses, and sap pressure, and thus may actively control the risk of xylem embolism (Jones and Sutherland 1991). The second objective of our study was to test the hypothesis of a stomatal control embolism during water stress in walnut.

Materials and Methods

Plant material and growth conditions

Experiments were conducted on 3-yr-old walnut trees (*Juglans nigra x regia* 'NG38' grafted on *Juglans regia* L. rootstocks) grown in 35 l pots containing 1/3 of peat and 2/3 of a natural clay soil from the Limagne region (Central France). The plants, grown in a nursery, were transferred to growth cabinets where air temperature (25°C) and air humidity (60% RH) were maintained constant. The light intensity at foliage level was adjusted between 0 and 480 µmol m^{-2} s^{-1}. A total of 17 trees were used in the different experiments. Because the experiments were time consuming we choused to combine multiple experiments with a rather limited number of repetitions.

Leaf Ψ and plant transpiration

Leaf water potentials (Ψ_{leaf}) were measured with a Scholander type pressure chamber on leaflets randomly sampled on the trees. The xylem water potential at the base of the leaflet

blades (Ψ_{xyl}) was measured on leaflets enclosed for at least two hours in an air proof aluminum bag (Turner and Long 1980).

Plant water loss (E_{plant}) was estimated with two independent techniques. Sap flow through the trunk was monitored by the heat balance technique of Valancogne and Nasr (1989). E_{plant} was also assessed gravimetrically with an electronic balance (model ID1, resolution 1g, Mettler, Viroflay, Switzerland). In addition, leaf transpiration and leaf stomatal conductance (g_s) were measured with a steady state porometer (model 1600 Li-Cor, Linclon, NE, USA). The measurements were performed on five leaflets randomly chosen in the trees.

Xylem embolism

The degree of xylem embolism due to the presence of air in the vessels was assessed following the procedure introduced by Sperry et al. (1988) and recently substantiate (Cochard et al. 2000). We used the new XYL'EM apparatus (Xylem Embolism Meter, INRA, Paris, France), based on a high-resolution liquid mass flowmeter, to determine the percentage of loss of hydraulic conductance (PLC) in the leaf rachis. Leaves were cut from the trees under tap water, and 15 to 20 mm long segments were excised, still under water, from the leaf rachis. The segments were attached to the tubing of the XYL'EM apparatus and their initial hydraulic conductance (K_{init}) determined with a hydrostatic pressure gradient of *ca* 3 kPa. The samples were then flushed with water pressurized to 0.1 MPa and the maximum conductance (K_{max}) determined as above. The PLC was calculated as follows:

$$PLC = 100 \times (1 - K_{init}/K_{max}) \qquad (1)$$

Whole plant hydraulic resistance

The whole plant hydraulic resistance (R_{plant}) was computed as the ratio between the water potential drop along the sap pathway and the whole plant transpiration:

$$R_{plant} = (\Psi_{soil}-\Psi_{xyl})/E_{plant} \qquad (2)$$

It should be noted that, as we have defined it, Rplant does not include the leaf blade hydraulic resistance.

Vulnerability curves

The vulnerability of the xylem conduits to air embolism was determined following the pressure chamber dehydration technique of Cochard et al. (1992). The technique consisted in inserting a plant segment in a pressure chamber with the cut end protruding. The pressure in the chamber was then adjusted to a target value and maintained constant until sap exudation ceased (usually after 15 to 30 min). The pressure was then released and xylem segments were excised under water and their PLC value determined as above. The plot of the PLC value versus the applied air pressure represents the "vulnerability curve" (VCs) of the plant segment. VCs were first obtained for leaflet midrib, leaf rachis and fine roots. A few measurements were also made on current year stem segments to confirm our previous results (Tyree et al. 1993).

For midrib and rachis VCs, entire leaves were pressurized. Four to six rachis segments were measured on each leaf. The basal 20-cm of the leaf rachis was discarded to avoid artifacts caused by air entry into cut vessels. On the same leaves, midribs were excised from the four apical leaflets. Root VCs were obtained on lateral roots excised from entire root systems gently washed with tap water. A total of 13 leaves and 13 roots from three different trees were used to construct the VCs. The following logistic function was fitted to the different curves (Cochard et al. 1999)

$$PLC(\Psi_{xyl})=100/(1+(\Psi_{xyl}/\ \Psi_{xyl50})^{s}) \quad (3)$$

where Ψ_{xyl50} is the water potential inducing 50 PLC and s is a slope parameter.

Soil dehydration

The stomatal response to a soil water deficit was studied on six different trees following two complementary procedures. The water deficit was induced by withholding irrigation. For the first procedure, the light intensity in the chamber was increased progressively during the day. Four light intensities (0, 170, 250, 450 µmol m^{-2} s^{-1}) were applied for two hours each. Plant transpiration was stabilized at the end of each period when Ψ_{leaf}, Ψ_{xyl} and g_s were determined. During the night, the light level was maintained at 170 µmol m^{-2} s^{-1} in order to progressively lower the soil water content. The procedure was repeated for four consecutive days for each of the three trees used in this experiment. In a second procedure, plants were continuously exposed to 450 µmol m^{-2} s^{-1} during seven consecutive days. Ψ_{leaf}, Ψ_{xyl} and g_s were determined twice a day. Leaves were periodically sampled for embolism assessment. The second procedure was repeated on three different trees.

Stem pressurization

The aim of this experiment was to analyze the stomatal response to an increase in trunk hydraulic resistance. We used the air injection technique (Cochard et al. 1992; Salleo et al. 1992) to induce air embolism in the trunk and thus increase the trunk resistance. A 20 cm long pressure chamber was fastened to the trunk of three different trees exposed to a constant light intensity (450 µmol m^{-2} s^{-1}). The pressure chamber was similar to the chamber described by Salleo et al. (1992), except that it was divided in two parts and could be clamped on intact trees. In order to facilitate air entry into the xylem conduits, six 1.3

mm diameter holes were drilled radially through the trunk inside the chamber (Améglio et al. 1994). The holes were aligned vertically in order to minimize the damage to the xylem conduits. In a preliminary experiment, we verified that fastening the pressure chamber to the tree had no effect on tree transpiration and Ψ_{leaf}. The trunk segment in the chamber was exposed to increasingly higher air pressures (up to 3.5 MPa). Each target pressure was maintained constant for 15 minutes. Two hours after each pressurization, two leaflets were sampled to measured Ψ_{xyl}, Ψ_{leaf} with a pressure chamber and g_s was measured on five leaflets. Leaves were also periodically sampled for embolism assessment.

Root chilling

This experiment aimed at altering root and soil hydraulic resistance by changing soil temperature (Brodribb and Hill 2000; Cochard et al. 2000). A well watered tree was placed in the growth chamber with 450 µmol m^{-2} s^{-1} light intensity. Ψ_{leaf}, Ψ_{xyl} and g_s were measured when E_{plant} became stable. Solutions with different temperature were obtained by mixing tap water with ice. The solutions were successively poured on the soil to lower its temperature (T_{soil}) in a step-wise manner from ambient to *ca* 0.5°C. T_{soil} was measured with a thermocouple inserted near the center of the pot. After *ca* 75 minutes Ψ_{leaf}, Ψ_{xyl} and g_s were measured and a cooler solution was poured on the soil. T_{soil} was then returned to 20°C by pouring water at ambient temperature on the soil. The whole procedure was repeated three times on two different trees.

Results

Vulnerability to cavitation

The VCs for the different walnut organs are shown in figure 1 and the parameters of the logistic fitting (equation 3) are given in table 1. Significant differences were found between organs. Leaf rachises were the most vulnerable, roots the least vulnerable and leaf veins and shoots intermediate. The few measurements obtained on shoot segments were in agreement with our previous study.

Soil dehydration

When plants were exposed to different light levels while soil watering was stopped (procedure 1), only a moderated water stress was induced. Figure 2 shows the concurrent changes in E_{plant} and Ψ_{xyl} for one tree. The other trees behaved the same. After four days, Ψ_{xyl} measured on the plant placed in the dark ("predawn" Ψ_{xyl}) was reduced only by 0.1 MPa (see *y* intercepts on figure 2). However, E_{plant}, g_s and the Ψ_{xyl} at maximum light intensity were considerably reduced at the end of the treatment. This was consistent with a sharp increase in whole plant hydraulic resistance (R_{plant}).

When plants were continuously exposed to a constant and high light intensity for one week (procedure 2), higher levels of water stress were obtained. Figure 3 shows the results for one tree, the other trees behaving the same. E_{plant} and g_s dropped close to zero while Ψ_{xyl} leveled at *ca* -1.4Mpa. At this point, the degree of xylem embolism in the leaf rachis was still less than 10PLC.

Root chilling

Decreasing soil temperature from ambient to near zero temperature provoked a significant reduction in g_s, E_{plant} and Ψ_{xyl} (figure 4). These changes were reversed when soil temperature was returned to ambient.

Stem pressurization

Application of a positive pneumatic pressure around the trunk of a plant induced a sharp decrease of E_{plant}, g_s and Ψ_{xyl} only for pressures above 2.5MPa (figure 5). At this pressure, about 70 PLC was induced in the stem (see the VC for stems plotted on top of figure 5). For air pressure applications higher than 3MPa, more than 95PLC was induced in the stem, leaf wilting was noticed and close to 100PLC was measured in the leaf rachis.

Discussion

The first objective of our study was to combine different experimental approaches to unravel the effect of plant hydraulics on stomatal closure. The second objective was analyze the effect of stomatal closure on plant hydraulics and to test the hypothesis of a stomatal control of embolism.

Unraveling the effects of Ψ_{soil}, R_{soil}, R_{root} and R_{shoot} in the response of g_s to water stress

In this study, we focused our analysis on some of the endogenous physiological parameters likely to be altered during a water stress and that have been reported in the literature to be associated with stomatal responses. These parameters are the soil water potential (Ψ_{soil}), the soil hydraulic resistance (R_{soil}), the root hydraulic resistance (R_{root}) and the shoot hydraulic resistance (R_{shoot}).

The effects of the different experiments conducted on the same plant material can be analyzed according to their impact on Ψ_{soil}, R_{soil}, R_{root} and R_{shoot}. Soil dehydration provoked a drop in both Ψ_{soil} and an increase of R_{soil}. The resistance of the interface between the soil and the root probably increased also (Nobel and Cui 1992). Rshoot was not altered because the level of xylem embolism remained low during these experiments. Root chilling altered R_{root} only because the temperature dependence of Ψ_{soil} is very low

(Muromtsev 1981). Stem pressurization provoked only an increase in R_{shoot} when the pressure exceeded the point of embolism induction (about 2.5MPa, Tyree et al. 1993 and see Figure 5). Therefore, combining the results of all these experiments, it is possible to determine if g_s and E_{plant} were specifically or not responding to changes in Ψ_{soil} , R_{soil} , R_{root} and/or R_{shoot}.

Our results showed that the different experiments all significantly reduced E_{plant} and g_s. Therefore, the response of g_s to Ψ_{soil}, R_{root}, R_{soil} and R_{shoot} was neither specific nor exclusive. An alternative analyze of the problem is not to consider Ψ_{soil}, R_{root}, R_{soil} and R_{shoot} individually but rather to examine their combining effect on Ψ_{xyl} or Ψ_{leaf}. The relationship between Ψ_{xyl}, Ψ_{soil}, R_{root}, R_{soil}, E_{plant} and g_s under steady-state conditions is well described by the Ohm's law analogy (Tyree and Ewers 1991):

$$\Psi_{xyl} = \Psi_{soil} + (R_{soil} + R_{root} + R_{shoot}).\ SF_{plant}\ .\ g_s\ .\ D \qquad (4)$$

where SF_{plant} is the plant leaf area and D the vapor pressure deficit, two parameters that remained constant during our experiments. A similar relationship is obtained with Ψ_{leaf} if we further include the leaf blade hydraulic resistance. The dependence of g_s and E_{plant} on Ψ_{xyl} is shown on figure 6 for all the experiments. The responses were similar whatever the experiments. Comparable relationships were obtained between E_{plant}, g_s and Ψ_{leaf} (not shown). This would suggest that, in these experiments, g_s was not correlated to changes in Ψ_{soil}, R_{soil}, R_{root}, or R_{shoot} *per se* but rather to their impact on Ψ_{xyl} and/or Ψ_{leaf}. This result is in agreement with the finding of Saliendra et al. (1995), Sperry (2000) or Hubbard et al. (2001). Our data do not enable us to test whether if g_s was more specifically responding to changes in Ψ_{leaf} or Ψ_{xyl} because both values were highly correlated and differed only by a few 0.1MPa. Experiments where Ψ_{xyl} and Ψ_{leaf} are uncoupled are awaited.

Evidence for a stomatal control of xylem embolism in walnut

From the above discussion, it could be concluded that the effect of the stomatal closure in our study was to maintain Ψ_{xyl} above a threshold value around –1.4 MPa. To further understand this behavior, we have to identify a major physiological trait that would threaten plant integrity at lower Ψ_{xyl} values. The answer to this question is obviously very complex, because many traits are probably involved and correlations between them probably exist. We will argue that xylem cavitation was a trait likely to explain stomatal closure in our study. A physiological trait responsible for a stomatal closure during water stress should meet at least the following three main conditions: first, its impairment should represent a serious threat to plant functioning. This results from the consideration that the cost associated with stomatal closure is high (reduced carbon gain, reduced growth, reduced reproductive success etc..) so the gain associated with the regulation should overcomes the lost. Cavitation is a serious threat for plants because it impairs the xylem conductive capacity and may eventually lead to leaf desiccation and branch mortality (Rood et al. 2000). Indeed, leaf desiccation was not observed in our study as long as the xylem integrity was maintained. Leaf desiccation was noticed only when high levels of embolism were measured in the leaf petioles. The gain associated with stomatal closure was thus the maintenance of leaf vitality, which largely overcomes the drawbacks cited above. The second condition is that the impairment of the trait should be water deficit dependent because the effect of stomatal closure is precisely to prevent excessive leaf dehydration. The mechanism of water-stress induced cavitation has been well documented (Sperry and Tyree 1988; Cochard et al. 1992; Jarbeau et al. 1995). Air is sucked into the xylem lumens through pores in the pit wall when pressures in the sap exceed the maximum capillary pressures that can sustain the pores. Therefore, the likelihood of cavitation occurrence is directly determined by the degree of water deficit in the xylem, more

precisely by Ψ_{xyl}. The third condition is that the impairment of the trait should have the same water deficit dependence than stomata. Stomata were completely closed in *Juglans* when Ψ_{xyl} reached about *ca* -1.4MPa and Ψ_{leaf} about *ca* -1.6MPa. The impairment of the trait associated with stomatal closure should therefore occurs at comparable Ψ_{xyl} values. On top of figure 6 representing E_{plant} versus Ψ_{xyl}, we have represented the vulnerability curve for leaf rachis (plain line). Leaf rachis was the most vulnerable organ along the sap pathway in the xylem and also exposed to the lowest Ψ_{xyl} values. Leaf rachis is therefore the Achille's heel of *Juglans* sap pathway. From figure 6, it is clear that the dependencies of leaf rachis xylem embolism and stomatal conductance on water deficit were very similar. To better quantify this dependence, we have conducted two series of calculations. First, we have computed the level of embolism in the leaf rachis that would have been reached if the stomata were not responding to the water stress (i.e. E_{plant} was kept constant and equal to its maximum value before treatment E_{max}). We have computed Ψ_{xyl} as:

$$\Psi_{xyl} = \Psi_{soil} + R_{plant} E_{max} \quad (5)$$

Reporting Ψ_{xyl} on the vulnerability curve yields the theoretical PLC value and the dotted line on figure 6. It can be seen that if stomata were maintained opened, very high degrees of embolism would been observed. This calculation shows that the control of embolism development probably imposed a limitation to E_{plant} and g_s even in the first stage of the water deficit.

Second, we have considered a much less drastic condition allowing the actual transpiration to be increased by 5% of E_{max}. In this case:

$$\Psi_{xyl} = \Psi_{soil} + R_{plant} (E_{plant} + 0.05E_{max}) \quad (6)$$

The predicted PLC values are also shown on figure 6 (dashed lines). Again, the predicted level of embolism would have been much higher if stomata were only slightly more open. This would suggest that the stomatal aperture was constantly adjusted during our

experiments to maximize E_{plant} (and photosynthesis) while minimizing PLC. In order to experimentally validate these computations, we have tried, without success, to feed stressed plants with fusiccocine, a drogue supposed to promote the stomatal opening. The use of mutants lacking efficient stomatal regulation is probably a better way to test such hypotheses (Cochard et al. 1996).

Mechanisms triggering stomatal closure

The mechanisms by which stomata could sense changes in Ψ_{xyl} to adjust g_s and avoid cavitation remain hypothetical. It seems improbable that Ψ_{xyl} itself was the triggering parameter so me must identify a parameter correlated to Ψ_{xyl} during water stress. Our stem pressurization experiments dismiss the implication of parameters associated with soil or root water stress (such as Ψ_{soil} or ABA production by roots (Tardieu et al. 1992)). Because the onset of cavitation events in leaf blades correlated with the onset of stomatal closure in Laurel, it has been that stomata were responding to hydraulic signals generated by cavitation (Salleo et al. 2000; Nardini et al. 2001). However, in our study stomatal closure occurred before the onset of cavitation in leaf blades and midribs (figure 6), and only after 70% loss of conductance in the trunk (figure 5). The possibility that stomata were responding to Ψ_{leaf} can not be rejected because of the high correlation between Ψ_{xyl} and Ψ_{leaf} in our study. Ψ_{leaf} could have a direct impact on guard cells turgor (Meidner and Edwards 1996) or an indirect impact on their sensibility to ABA (Tardieu and Davis 1993).

Conclusion

In conclusion, combining different experimental procedures, we have established that stomata were not responding to changes in Ψ_{soil}, R_{soil}, R_{root}, or R_{shoot} *per se* but rather to their impact on Ψ_{xyl} or Ψ_{leaf}. Cavitation avoidance was a likely physiological function associated with stomatal regulation during water stress in these experiments. The significance of cavitation avoidance seems crucial to understand plant water relations during water stress, probably more than the occurrence of cavitation itself. The possibility still exists that the striking relations that have been observed between cavitation and stomatal function are only correlations and that the main physiological trait involved in the regulation is elsewhere. However, it seems to us that cavitation avoidance fits the three conditions we have identified for such a trait better that any other trait so far.

References

Améglio T, Cruiziat P, Bodet C (1994) A pressure chamber to induce and control embolism in situ: consequence on the sap flow. In: Kouchkovsky Y, Larcher F (eds) Plant Sciences. SFPV, Paris, pp 281

Brodribb TJ, Hill RS (2000) Increases in water potential gradient reduce xylem conductivity in whole plants. Evidence from a low-pressure conductivity method. Plant Physiol 123: 1021-1028.

Cochard H, Cruiziat P, Tyree MT (1992) Use of positive pressures to establish vulnerability curves. Further support for the air-seeding hypothesis and implications for pressure-volume analysis. Plant Physiol 100: 205-209

Cochard H, Bréda N, Granier A (1996) Whole tree hydraulic conductance and water loss regulation in *Quercus* during drought. Evidence for stomatal control of embolism ? Ann Sci Forest 53: 197-206

Cochard H, Ridolfi M, Dreyer E (1996) Responses to water stress in an ABA-unresponsive hydrid poplar (*Populus koreana x trichocarpa* cv. Peace) II. Hydraulic properties and xylem embolism. New Phytol 134: 455-461

Cochard H, Lemoine D, Dreyer E (1999) The effects of acclimation to sunlight on the xylem vulnerability to embolism in *Fagus sylvatica* L. Plant Cell Environ 22: 101-108

Cochard H, Bodet C, Améglio T, Cruiziat P (2000) Cryo-scanning electron microscopy observations of vessel content during transpiration in walnut petioles. Facts or artifacts? Plant Physiol 124: 1191-1202

Cochard H, Martin R, Gross P, Bogeat-Triboulot MB (2000) Temperature effects on hydraulic conductance and water relations of *Quercus robur* L. J Exp Bot 51: 1255-1259

Hinckley TM, Bruckerhoff DM (1975) The effects of drought on water relations and stem shrinkage of *Quercus alba*. Can J Bot 53: 62-72

Hubbard RM, Ryan MG, Stiller v, Sperry JS (2001) Stomatal conductance aand photosynthesis vary linearly with plant hydraulic conductance in ponderosa pine. Plant Cell Environ 24: 113-121

Jarbeau JA, Ewers FW, Davis SD (1995) The mechanism of water-stress-induced embolism in two species of chaparral shrubs. Plant Cell Environ18: 189-196

Jones HG, Sutherland RA (1991) Stomatal control of xylem embolism. Plant Cell Environ 14: 607-612

Kramer PJ, Boyer JS (1995) Water relations of plants and soils. Academic Press.

Lu P, Biron P, Granier A, Cochard H (1996) Water relations of adult Norway spruce (*Picea abies* L Karst) under soil drought in the Vosges mountains. Whole-tree hydraulic conductance, xylem embolism and water loss regulation Ann Sci Forest 53: 113-121

Meidner H, Edwards M (1996) Osmotic and turgor pressures of guard cells. Plant Cell Environ19 : 503

Meinzer FC, Grantz DA (1990) Stomatal and hydraulic conductance in growing sugarcane. Stomatal adjustment to water transport capacity. Plant Cell Environ 13: 383-388

Muromtsev NA (1981) Effects of temperature on the potential of soil water and the availability to plants. Soviet Meteorology Hydrology 5: 68-72

Nardini A, Tyree Mt, Salleo S (2001) Xylem cavitation in the leaf of Prunus laurocerasus and its impact on leaf hydraulics. Plant Physiol 125: 1700-1709

Nobel PS, Cui MY (1992) Hydraulic conductances of the soil, the root soil air gap, and the root. Changes for desert succulents in drying soil. J Exp Bot 43: 319-326

Pickard WF (1981) The ascent of sap in plants. Prog Biophys Mol Biol 37: 181-229

Rood SB, Patino S, Coombs K, Tyree MT (2000) Branch sacrifice: cavitation-associated drought adaptation of riparian cottonwoods. Trees 14: 248-257

Saliendra NZ, Sperry JS, Comstock JP (1995) Influence of leaf water status on stomatal response to humidity, hydraulic conductance, and soil drought in *Betula occidentalis*. Planta 196: 357-366

Salleo S, Hinckley TM, Kikuta SB, Lo gullo MA, Weilgony P, Yoon TM, Richter H (1992) A method for inducing xylem emboli in situ : experiments with a field-grown tree. Plant Cell Environ 15: 491-497

Salleo S, Nardini A, Pitt F, Lo Gullo MA (2000) Xylem cavitation and hydraulic control of stomatal conductance in laurel (Laurus nobilis L.). Plant Cell Environ 23 :71-79.

Schulze ED, Turner NC, Gollan T, Shackel KA (1987) Stomatal responses to air humidity and to soil drought. In: Zeiger Z, Farquhar GD, Cowan IR (Eds), Stomatal function. Stanford Univ. Press, Stanford, Calif., USA, pp 311-321.

Sperry JS, Donnelly JR, Tyree MT (1988) A method for measuring hydraulic conductivity and embolism in xylem. Plant Cell Environ 11: 35-40

Sperry JS, Tyree MT (1988) Mechanism of water stress-induced xylem embolism. Plant Physiol 88: 581-587

Sperry JS, Alder NN, Eastlack SE (1993) The effect of reduced hydraulic conductance on stomatal conductance and xylem cavitation. J Exp Bot 44: 1075-1082

Sperry JS (2000) Hydraulic constraints on plant gas exchange. Agr Forest Meteorol 104: 13-23

Tardieu F, Zhang J, Davies WJ (1992) What information is conveyed by an ABA signal from maize roots in drying field soil. Plant Cell Environ 15 :185-191.

Tardieu F, Zhang J, Gowing DJG (1993) Stomatal control by both [ABA] in the xylem sap and leaf water status - A test of a model for droughted or ABA-fed field grown maize. Plant Cell and Environ 16 : 413-420.

Turner NC, Long MJ (1980) Errors arising from rapid water loss in the measurement of leaf water potential by the pressure chamber technique. Aust J Plant Physiol 7 : 527-237

Tyree MT, Sperry JS (1988) Do woody plants operate near the point of catastrophic xylem dysfunction caused by dynamic water stress ? Answers from a model. Plant Physiol 88: 574-580

Tyree MT, Ewers FW (1991) The hydraulic architecture of trees and other woody plants. New Phytol 119: 345-360

Tyree MT, Cochard H, Cruiziat P, Sinclair B, Améglio T (1993) Drought-induced leaf shedding in walnut. Evidence for vulnerability segmentation. Plant Cell Environ 16: 879-882

Valancogne C, Nasr Z (1989) A heat balance method for measuring sap flow in small trees. In: Borghetti M, Grace J, Raschi A (eds) Water transport in plants under climatic stress. Cambridge University Press, Cambridge, pp 166-173

Zeiger E, Farquhar GD, Cowan IR (1987) Stomatal function. Stanford University Press, Stanford, USA. 503 p.

Captions

Figure 1: Vulnerability curves for *Juglans regia x nigra* leaf midribs and rachis, and current yr. shoots and roots. Close symbols refer to measures on air-pressurized segments. Open symbols refer to leaf midribs and rachis collected on trees during the different experiments. Error bars are ± SE. Lines are logistic fits through the data. For shoots, the lines are logistic fits through the data published by Tyree et al. (1993) for current yr. (plain line) and previous yr. (dashed line) segments.

Figure 2: Typical changes of plant transpiration and xylem water potential during the first stage of a soil water stress for one of the studied tree. The four different symbols correspond to four consecutive days. Four different light intensities were used on each day to vary E_{plant}. Lines are linear regressions through the data for each day. The slope of the lines represents the whole plant hydraulic resistance (R_{plant}).

Figure 3: Typical time course of plant transpiration and xylem water potential for a non irrigated tree exposed to a constant light intensity.

Figure 4: Typical time course of xylem water potential and soil temperature (upper panel), and plant transpiration and stomatal conductance (lower panel) during a soil chilling experiment. Error bars are ± SE (n=5).

Figure 5: Changes in stomatal conductance (upper panel) and xylem water potential (lower panel) of trees exposed to increasing pneumatic pressures around their trunk (x axis). The curve represents the change in embolism in the trunk versus the applied pressure.

Figure 6: Dependence of stomatal conductance (upper panel) and transpiration (E_{plant}) on the xylem water potential. The different symbols represent the different experiments conducted in this study. Transpiration was normalized by the transpiration of each tree before treatment (E_{plant} /E_{max}). The plain curve represent the vulnerability curve for the leaf rachis. The dotted and dashed curves are the predicted level of embolism if E_{plant} was maintain to E_{max} and E_{plant} + 0.05 E_{max} respectively.

Table 1: Xylem vulnerability to embolism. Parameters of the logistic function (see equation in the text) fitted to the experimental PLC versus Ψ_{xyl} curves. Values are given ± SE. Data having a letter in common are not significantly different at P = 0.01.

	Leaf		Shoot		Root
	Midrib	Rachis:	year n	year n-1	
Ψ_{xyl50}	-2.12±0.06a	-1.72±0.05b	-2.10±0.04a	-2.36±0.02c	-3.53±0.39d
s	-9.77±2.00ac	-5.72±0.88abd	-9.82±1.97bc	-12.8±1.95c	-3.50±1.31d

Figure 1

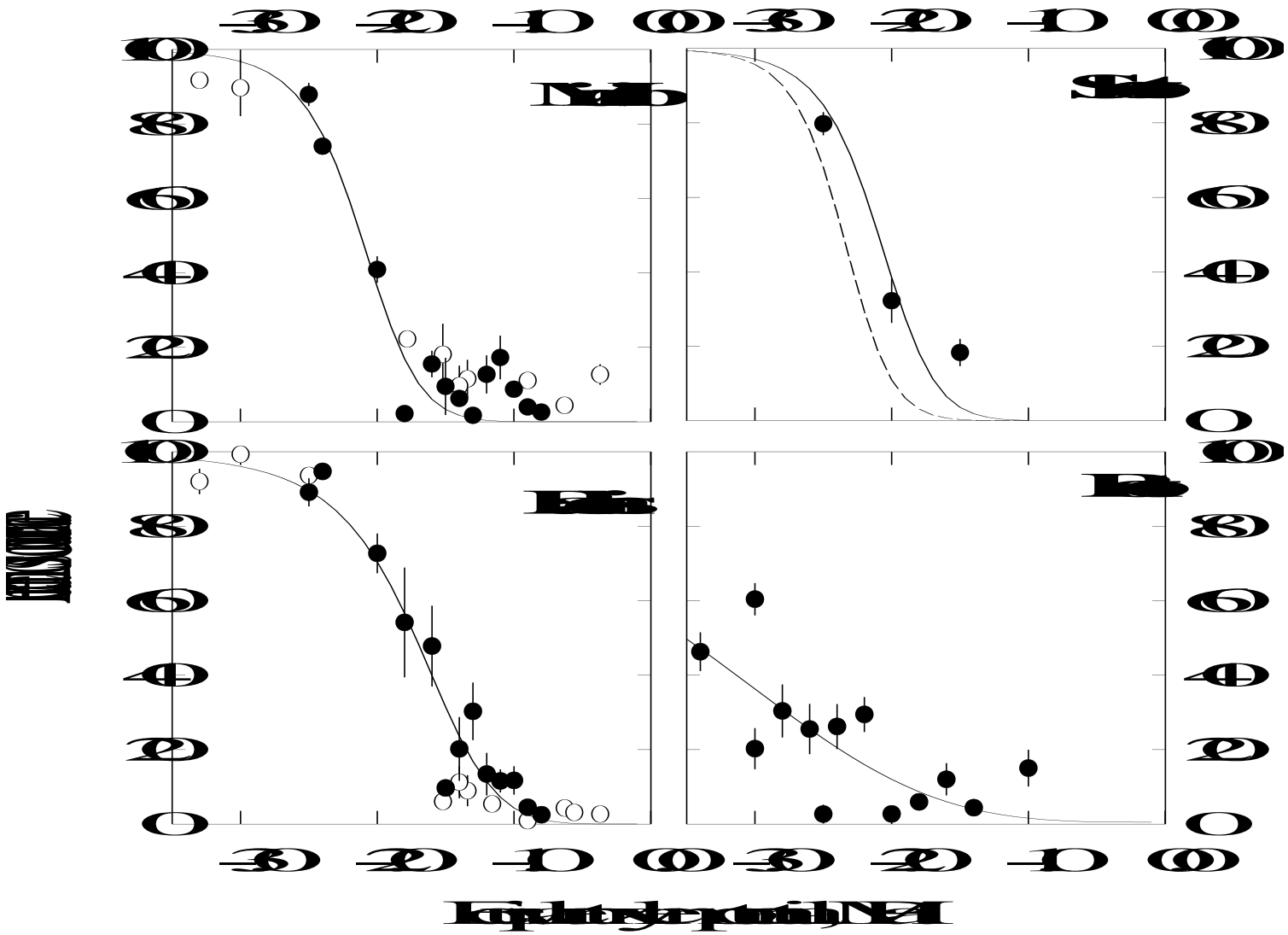

Figure 2

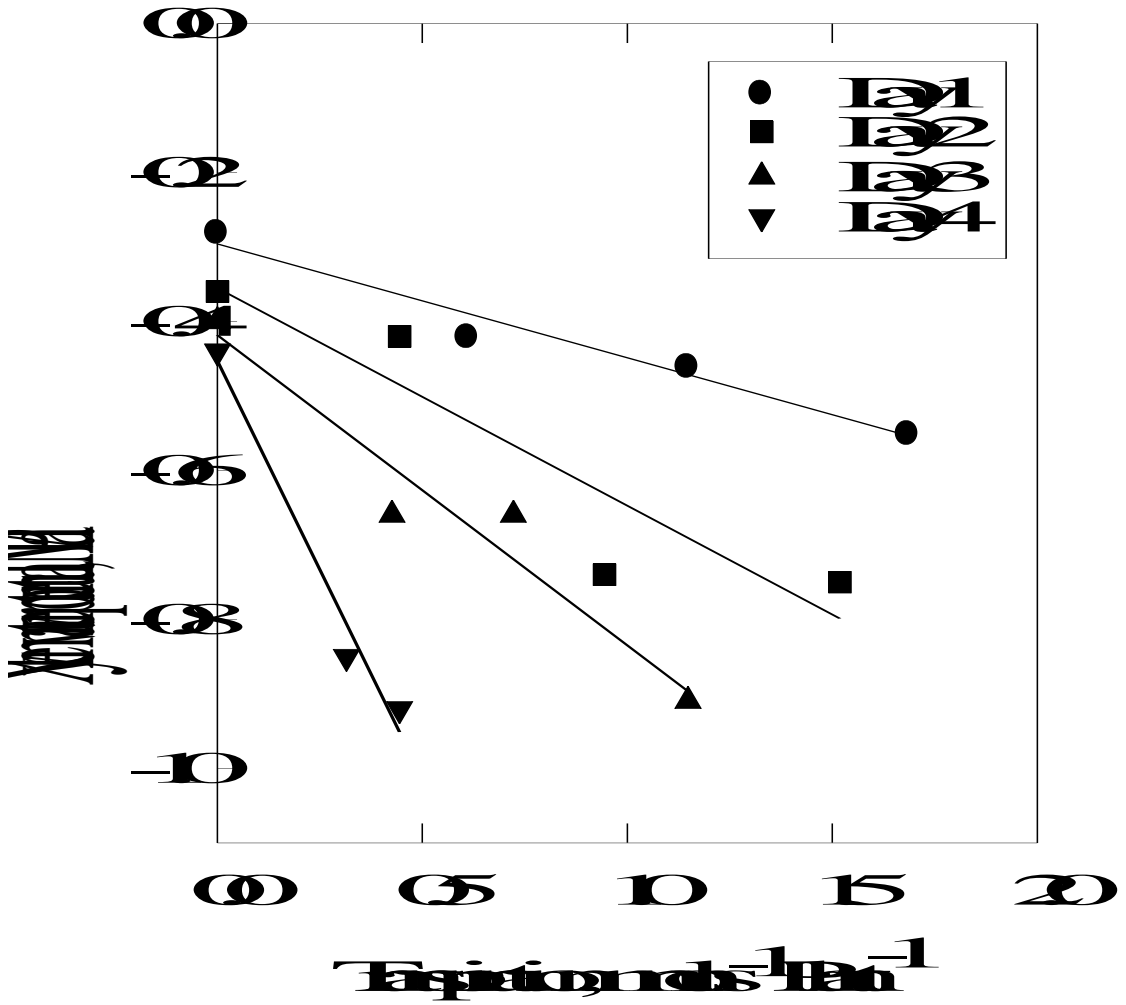

Figure 3

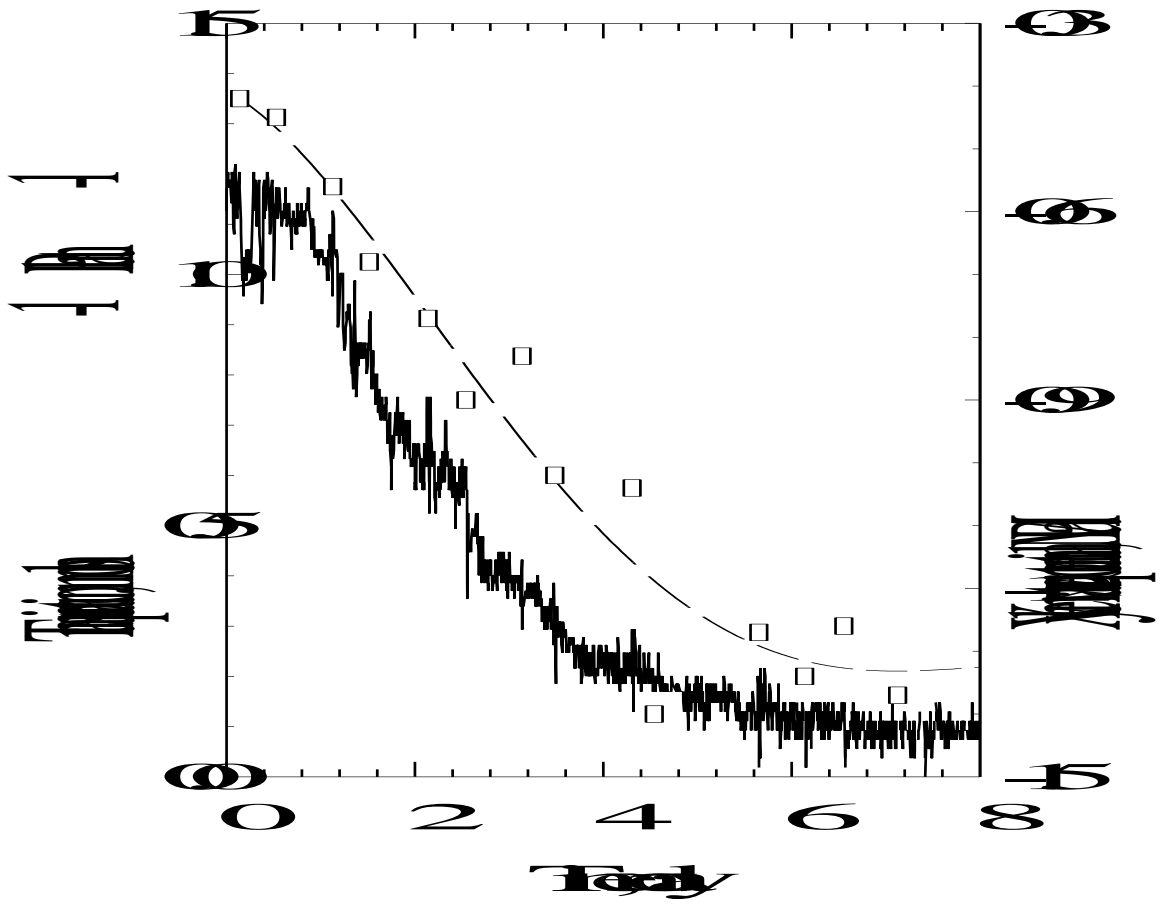

Figure 4

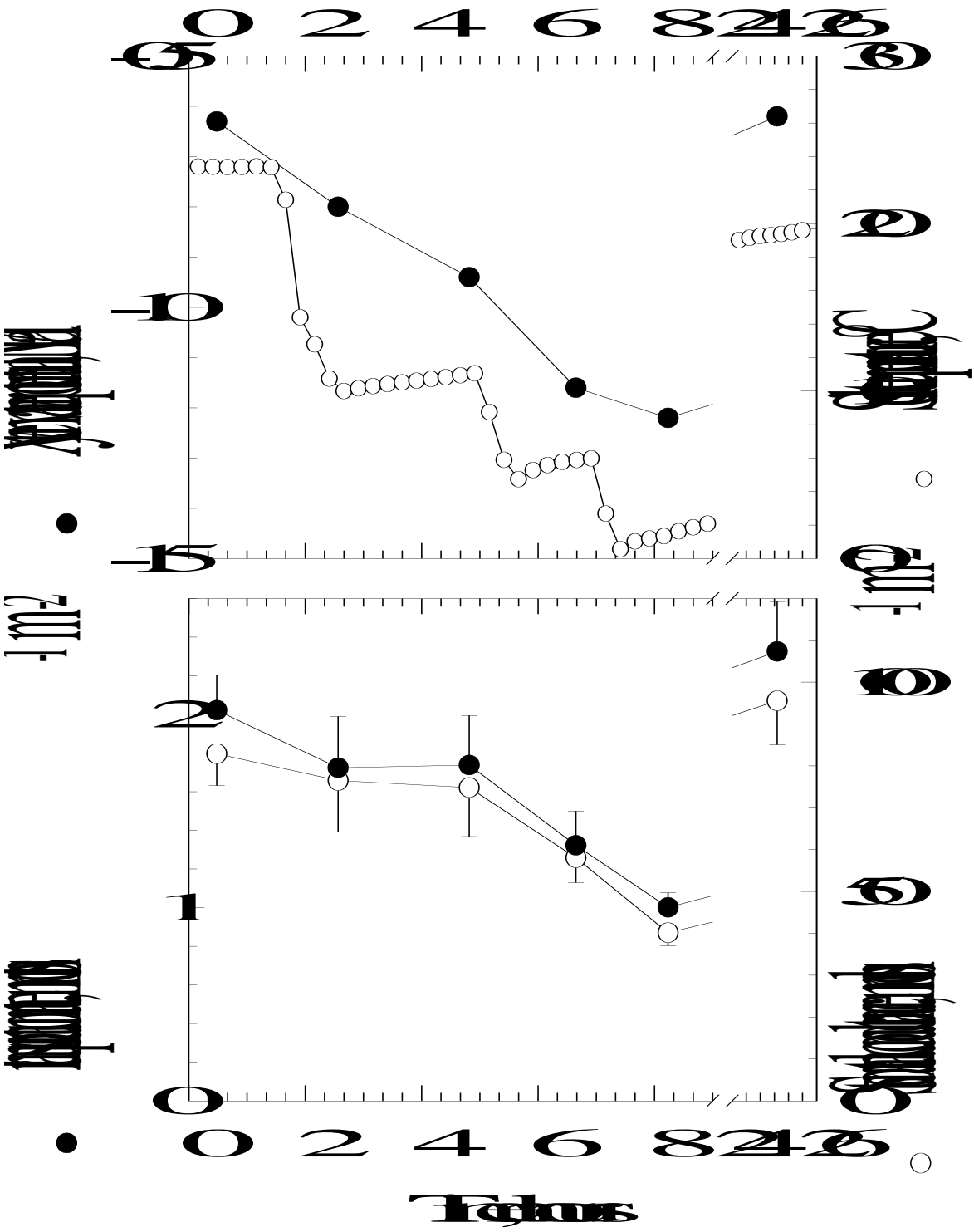

Figure 5

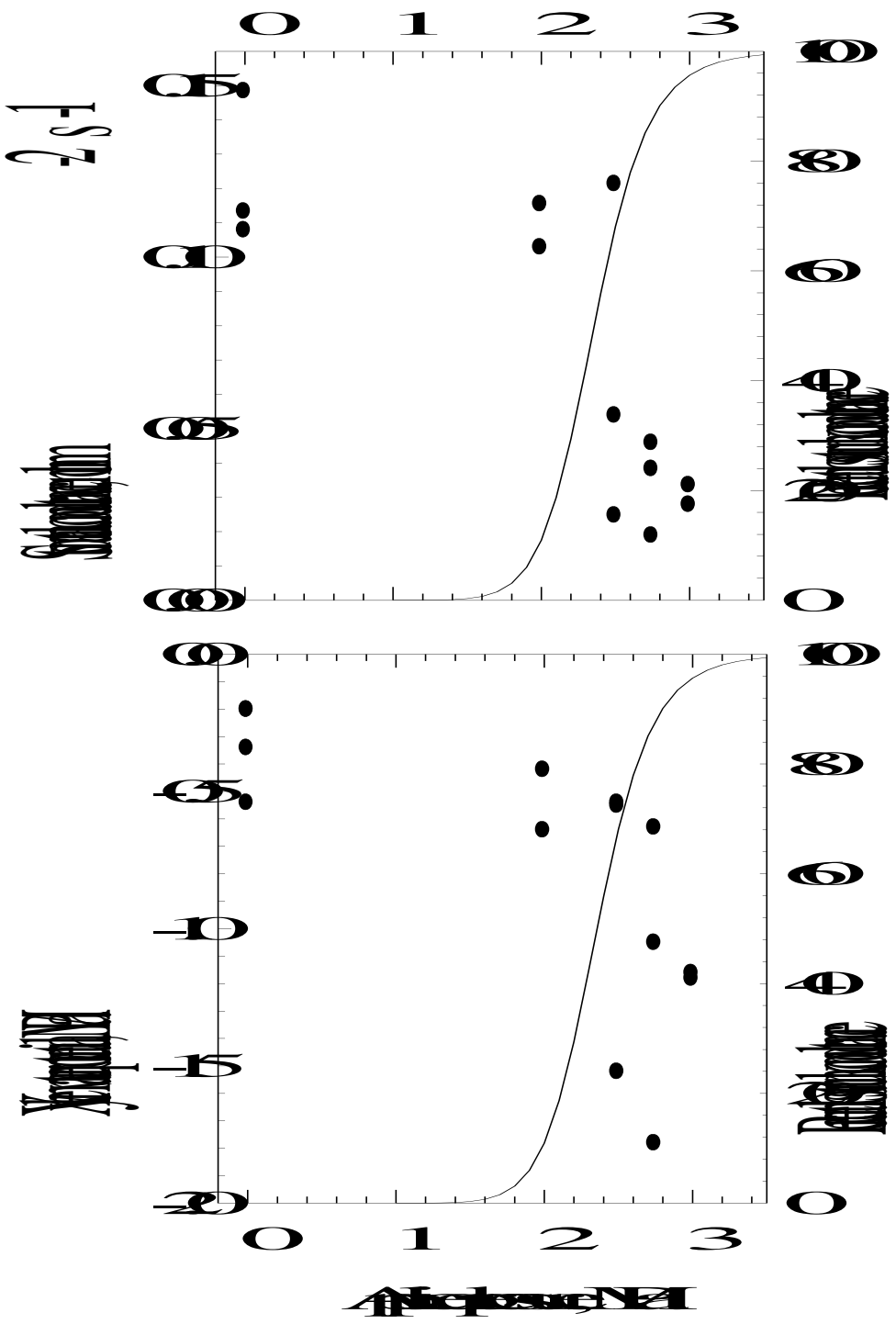

Figure 6